Plant Physiol. (1981) 67, 1078–1083
0032-0889/81/67/1078/06/$00.50/0

White Light Effects on the mRNA for the Light-Harvesting Chlorophyll *a/b*-Protein in *Lemna gibba* L. G-3[1]

Received for publication March 31, 1980 and in revised form November 24, 1980

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ABSTRACT

Translation products of poly(A) mRNA isolated from *Lemna gibba* L. G-3 include a major polypeptide of 32,000 daltons which is immunoprecipitated by antiserum to chlorophyll *a/b*-protein from *Chlamydomonas*. This 32,000 dalton polypeptide represents a precursor to the light-harvesting chlorophyll *a/b*-protein of molecular weight 28,000 found in the thylakoid membranes of *Lemna gibba*. The amount of this translatable mRNA decreases relative to other translatable mRNAs when green plants grown in continuous white light are placed in darkness. This decrease occurs rapidly. The most rapid decline occurs during the first day; after 4 days of darkness, only a low level of this mRNA can be detected by *in vitro* translation. When the plants are returned to white light there is an increase in the relative level of this mRNA which can be easily detected within two hours. The *in vivo* synthesis of this protein has been assayed under the different light conditions. The light effects on the *in vivo* synthesis of the chlorophyll *a/b*-protein reflect the light effects on the translatable mRNA for the polypeptide. The results indicate that light induced changes in the synthesis, processing, or degradation of chlorophyll *a/b*-protein mRNA could account for the light-induced changes observed in the effective synthesis rates for the chlorophyll *a/b*-protein *in vivo*.

Light is involved in the regulation of many aspects of the growth and development of higher plants both at the whole plant and at the cellular level. One of the major cellular events which is affected by light is the synthesis and development of an active photosynthetic apparatus. In *Lemna gibba*, an aquatic plant, the synthesis of the major soluble chloroplast protein, RuBP[2] carboxylase, can be regulated by light (24, 26). There are many other examples of species of higher plants in which the synthesis of this protein is affected by light (10, 11, 12, 15, 19).

We have shown (24, 26) that in *L. gibba* the effects of light and dark on the synthesis of RuBP carboxylase are due in part to their effect on the level of translatable mRNA coding for the small subunit of the enzyme. The amount of translatable mRNA coding for the precursor of the small subunit of RuBP carboxylase decreases relative to other mRNAs when light-grown plants are moved into the dark. Returning the plants to white light results in a rapid increase in the detectable level of this mRNA. Furthermore, we observed that the synthesis of the small subunit of RuBP carboxylase *in vivo* reflected the amount of mRNA for the protein which could be detected by the *in vitro* translation assay (26). I concluded that the light effect on the level of translatable mRNA for the small subunit of RuBP carboxylase could account for its effect on the synthesis of the protein. In the initial study, Tobin (24) also observed that a second abundant mRNA species was affected by the light-dark regime in a similar way to the RuBP carboxylase small subunit mRNA. This second mRNA might be the mRNA for the light-harvesting Chl *a/b*-protein (24). In this report I present the evidence that this second mRNA species is indeed that coding for the Chl *a/b*-protein. The synthesis of this protein and the level of its translatable mRNA in *L. gibba* respond to light in a manner similar to what was found for the small subunit of RuBP carboxylase. Apel and Kloppstech (2) have also presented evidence for a light-induced appearance of translatable mRNA for the Chl *a/b*-protein in etiolated barley, and Bennett (personal communication) has studied such an effect in pea.

MATERIALS AND METHODS

The methods referred to below have been described in greater detail in our earlier work (24–26). *L. gibba* L. G-3 were grown aseptically under continuous light (24). Poly(A) RNA was isolated from polysomal pellets by oligo(dT)-cellulose chromatography (24) and translated in an *in vitro* protein-synthesizing system from wheat germ (20). The ranges of poly(A) RNA and polysomal RNA recovered from *Lemna* over many experiments are 0.5 to 1.2 μg/g fresh weight and 20 to 70 μg/g fresh weight, respectively. The recovery showed no dependence on conditions of illumination. Because of this large variation in recovery of RNA, all comparisons of different samples are made on a relative rather than an absolute basis (mRNA as percentage of total mRNA). The *in vitro* translations were carried out on 1 to 2 μg poly(A) RNA in 50 μl reaction mixtures by using high specific activity [^{35}S]methionine (800–1,400 Ci/mmol, Amersham) for 90 min at room temperature.

Antiserum against the Chl *a/b*-protein from *Chlamydomonas reinhardtii* was a generous gift of Dr. I. Ohad, Hebrew University, Jerusalem (21). Immunoprecipitation of a specific polypeptide from the translation products was carried out by the *Staphylococcus aureus* technique (13, 14). *S. aureus* cells (Cowan 1 strain) were obtained as IgGsorb from The Enzyme Center, Boston, MA and used as a 10% suspension of the cells. Appropriate aliquots (15–48 μl) of translation products were added to 250 μl of TNT buffer. This solution was incubated with 15 μl of control or immune serum for 30 to 60 min at room temperature. The antibody-antigen complex was precipitated by addition of 120 μl of freshly washed IgGsorb and incubated at room temperature for 30 min. The precipitate was washed five to eight times with TNT, and dissociated from the *Staphylococcus* cells by boiling in 50 μl TNT containing 2.0% SDS and 6 M urea. Because this technique does not depend on forming a precipitable antigen-antibody complex, it is especially suitable for detecting the small amounts of radioactive product present in the translation products. The relative amounts of immunoprecipitates and translation products were determined after electrophoretic separation by densitometer scans

[1] This research was supported by National Institutes of Health Grant GM-23167 and by the University Research Committee.

[2] Abbreviations: RuBP, ribulose, 1,5-bisphosphate; TNT buffer, 50 mM Tris-Cl (pH 7.6), 0.1 M NaCl, 0.1% Triton X-100.

of preflashed and appropriately exposed fluorograms (16). To show that the amount of immunoprecipitable Chl *a/b*-protein recovered from the *in vitro* translation products is related linearly to the amount of mRNA for the protein added to the system, *Lemna* poly(A) RNA was mixed with chicken erythrocyte poly(A) RNA in varying proportions and the mixture translated in the wheat germ system. The trichloroacetic acid precipitable radioactivity present in the total translation products and in the immunoprecipitates was measured for each sample, and the immunoprecipitates were also characterized by electrophoresis and fluorography.

In vivo labeling of newly synthesized proteins was assayed by incorporation of [^{35}S]methionine as was previously described (26). In these studies the intact plants were labeled for 1 h with [^{35}S]-methionine (New England Nuclear or Amersham) in the light or dark as appropriate. The membrane fraction was separated from the soluble fraction by centrifugation, and solubilized by heating in the grinding buffer with the addition of 1% SDS (7, 26). The Chl *a/b*-apoprotein was identified in several ways. Unheated membrane fractions were electrophoresed on discontinuous 12.5% polyacrylamide-SDS gels (24) and the green band corresponding to the Chl *a/b*-protein (23) was excised, soaked in SDS-Tris-glycine sample buffer (26), and heated. This band was then reelectrophoresed on another 12.5% gel and the gel stained for protein with Coomassie Blue. Secondly, this protein was immunoprecipitated from the SDS-solubilized membrane fraction by using the antiserum from *Chlamydomonas* and the *S. aureus* procedure. The immunoprecipitation procedure was identical to that followed for the translation products except that the antigen-antibody complex was allowed to form in the presence of 2.5% Nonidet P-40 instead of 0.1% Triton X-100. The increased concentration of the detergent serves to keep the SDS from interfering with the immunoprecipitation (Dr. N.-H. Chua, The Rockefeller University, personal communication).

All lanes shown in a figure were electrophoresed on the same gel. All lanes shown and compared on fluorograms were exposed for the same amount of time to the x-ray film.

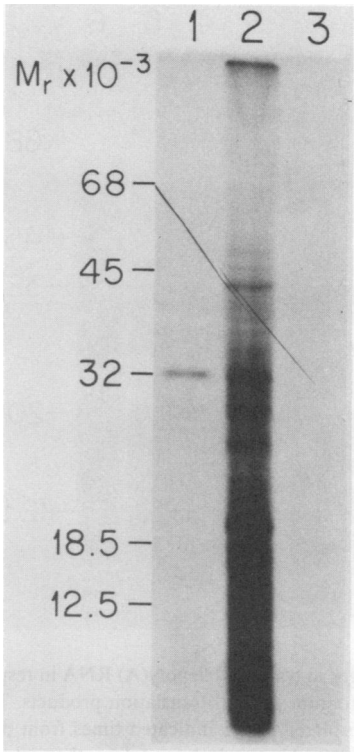

FIG. 1. Immunoprecipitation of the precursor of the Chl *a/b*-protein from wheat germ translation products of poly(A) RNA isolated from light-grown plants. Fluorogram of translation products and immunoprecipitate after electrophoresis on a 12.5% polyacrylamide gel. 1, immunoprecipitate from an aliquot of the translation products; 2, total translation products; 3, control immunoprecipitate using nonimmune serum with an aliquot of the translation products.

RESULTS AND DISCUSSION

The Chl *a/b*-protein is well known to be the major chloroplast membrane polypeptide (5, 23). The major membrane polypeptide in *L. gibba* is a doublet with a mol wt of about 28,000 (Figs. 5 and 6). I could confirm that this doublet includes the apoprotein of the Chl *a/b*-protein by isolating the green complex on a 12.5% polyacrylamide gel. On such a gel the complex runs with a lower mobility than the apoprotein. After dissociating the complex and electrophoresing the resulting polypeptides, one or more of which must have been associated with the Chl, it can be seen that the major polypeptide doublet from this Chl *a/b*-complex corresponds in mobility to the major membrane polypeptide (data not shown). In addition, we have used the antiserum to the Chl *a/b*-protein from *Chlamydomonas* to immunoprecipitate the apoprotein doublet from the solubilized membranes of *Lemna* (see below).

The Chl *a/b*-protein precursor polypeptide was identified in translation products coded for by mRNA isolated from *L. gibba* by using the antiserum to this protein from *Chlamydomonas* (21). A single band of mol wt 32,000 was precipitated from the translation products of *L. gibba* poly(A) RNA (Fig. 1). This polypeptide represents one of the major translation products in the system. In contrast, Chua and Schmidt (9) have reported that two bands (of 32,000 and 34,000 daltons) can be immunoprecipitated by antiserum to a Chl *a/b*-protein from *Chlamydomonas* from spinach poly(A) RNA *in vitro* translation products. Recently, I have used a sample of Dr. N.-H. Chua's antiserum to immunoprecipitate *Lemna in vitro* translation products and found that there is also a second minor immunoprecipitate band at about 31,000 daltons. These immunoprecipitated translation products presumably represent precursors to the 28,000 dalton Chl *a/b*-apoprotein doublet found *in vivo* in *Lemna*. Other workers (2, 9; J. Bennet, personal communication) using other species have also found that this protein is synthesized as a larger molecular weight form which must be processed to the smaller protein isolated from chloroplast membranes. The exact relationship between the precursors and the apoproteins is not known. For the purposes of this work I refer to the apoprotein doublet as the Chl *a/b*-apoprotein.

The effect of light on the level of the translatable mRNA for the Chl *a/b*-protein was investigated by *in vitro* translation. Figure 2 shows the changes occurring in the translatable mRNA for the Chl *a/b*-protein relative to the other poly(A) mRNAs when plants were put into the dark, then back out into the light for the times indicated before isolating the poly(A) RNA. The *in vitro* wheat germ system was programmed with approximately 2 μg of poly(A) RNA from each sample. Nearly equal amounts of acid-precipitable counts were loaded in each lane. The location of the Chl *a/b*-protein precursor at 32,000 daltons is marked in the figure. It can easily be seen that: (a) the amount of this protein decreases relative to the other translation products from RNA samples isolated from dark-treated plants (lanes 1–3); and (b) returning the plants to light results in a rapid increase in the level of this translatable mRNA (lanes 4–6). Because the exposure of the fluorogram was optimized for the Chl *a/b*-protein, the precursor to the small subunit of RuBP carboxylase at 20,000 daltons is overexposed in some of the samples. This overexposure makes it difficult to see in this figure the previously reported (26) changes in the translatable mRNA for this protein.

The changes in the amount of translatable mRNA for the Chl *a/b*-protein relative to other proteins can be more quantitatively measured by immunoprecipitation. Figure 3 demonstrates that the

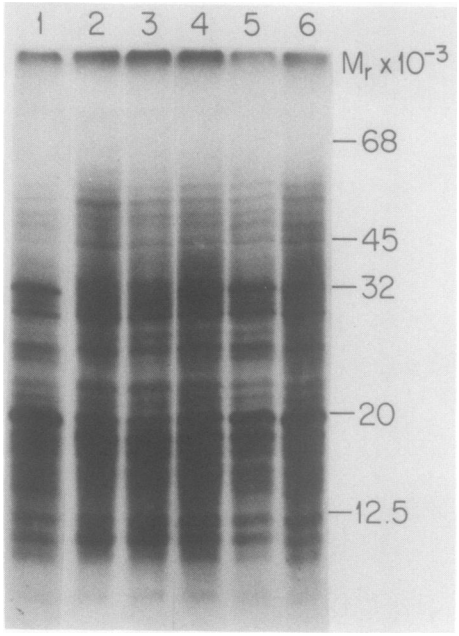

FIG. 2. Changes in translatable poly(A) RNA in response to dark/light treatments. Fluorogram of total translation products. The RNA used for translations was isolated at the indicated times from plants grown under continuous light, placed in the dark for 4 days, then returned to continuous light. 1, continuous light; 2, 1 day dark; 3, 2 days dark; 4, 4 days dark + 2 h light; 5, 4 days dark + 5 h light; 6, 4 days dark + 10.7 h light. Acid precipitable radioactivity in cpm $\times 10^{-3}$ loaded onto lanes: 1, 78.0; 2, 80.7; 3, 79.0; 4, 79.9; 5, 78.3; 6, 80.6.

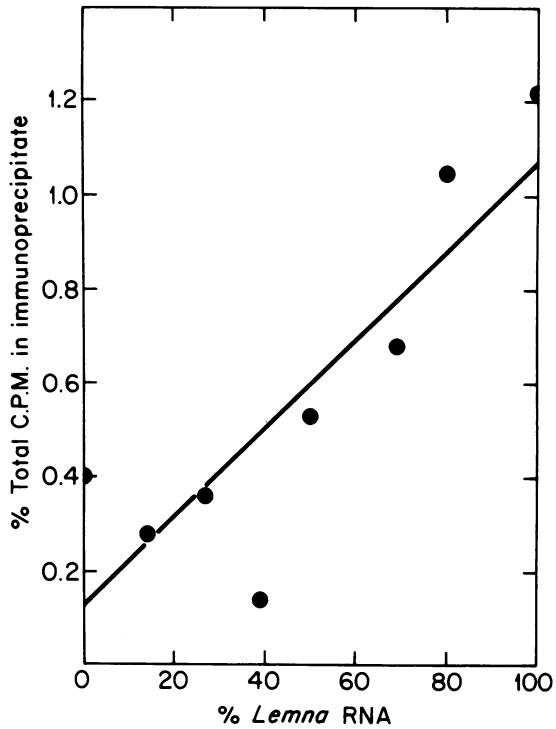

FIG. 3. Immunoprecipitable ^{35}S-labeled Chl *a/b*-protein in translation products as a function of varying concentrations of *Lemna* poly(A) RNA in a mixture with erythrocyte poly(A) RNA. Radioactivity recovered in the immunoprecipitate was calculated as the per cent of total acid precipitable radioactivity in the translation products. Amounts of *Lemna*/erythrocyte poly(A) RNA used in each translation mix for the data shown (μg): 0/1.00, 0.15/.090, 0.30/0.80, 0.45/0.70, 0.60/0.60, 0.90/0.40, 1.20/0.20, 1.50/0. Range of total acid precipitable counts in 48 μl translation products: 5.3 to 8.0 $\times 10^5$ cpm. Range of immunoprecipitable counts in 48 μl translation products: 1080 to 6424 cpm.

recovery of Chl *a/b*-protein precursor in the immunoprecipitate is proportional to the amount of its mRNA used in the *in vitro* wheat germ system. In this experiment the *Lemna* poly(A) RNA was mixed with erythrocyte poly(A) RNA in order to vary the amount of mRNA for the Chl *a/b*-protein relative to other mRNAs as well as in absolute amount. The amounts of each type of mRNA used were adjusted to result in a reasonably consistent amount of incorporation of radioactivity into translation products. The erythrocyte RNA incorporated more radioactivity/μg than the *Lemna* RNA. The results are presented as the per cent radioactivity incorporated to compensate for the differences in the total counts in translation product for each sample. Results similar to those shown in this figure have also been obtained with mixtures of pea leaf mRNA and *Xenopus laevus* ovary mRNA (A. Cuming and J. Bennett, personal communication). Thus, it is possible to use the immunoprecipitation of the translation products to show specifically and quantitatively the changes in the poly(A) RNA coding for the Chl *a/b*-protein seen in Figure 2.

The results of such an immunoprecipitation experiment are presented in Figure 4 and Table I. As in the earlier experiment, plants were grown in continuous light, transferred to the dark for 4 days, then returned to continuous light. Samples were removed at various times and the poly(A) RNA isolated and translated. Aliquots of translation products containing approximately equal total amounts of radioactivity (29–32 $\times 10^3$ cpm) were used to obtain the immunoprecipitates shown in lanes 1 to 8 (Fig. 4). Therefore, the densitites of the bands seen on this fluorogram represent the relative amounts of this protein in the total labeled translation products for each sample of RNA. When *L. gibba* is grown in continuous light and then transferred to the dark, the amount of translatable poly(A) RNA coding for the precursor of the Chl *a/b*-protein declines rapidly and for a period of at least 4 days (lanes 1–4) (Fig. 4). When the plants are returned to light the amount increases within a few hours (lanes 5–7). Densitometer scans were made of the fluorogram (16) and the relative amounts of immunoprecipitated Chl *a/b*-protein were estimated by cutting out and weighing the peak (Table I). The changes observed occur rapidly. After 1 day of darkness, only about 11% of the initial level of the poly(A) mRNA for the Chl *a/b*-protein can be detected. Two hours after returning the dark-treated plants to white light, about 15 times more Chl *a/b*-protein can be detected in the translation products of isolated mRNA than is present in the sample immediately before exposure to light, *i.e.* from plants given 4 days of darkness (*cf.* lanes 4 and 5, Fig. 4). The amount of Chl *a/b*-protein mRNA seems to be lower 10 h after returning the plants to light than it is at 5 h (*cf.* lanes 6 and 7). We have seen this pattern in two separate experiments, as well as in repeated translations of the poly(A) RNA from these samples, but the significance of this observation is unknown. Apel and Kloppstech (2) also observed a rapid increase (detectable within 2–3 h) in the translatable mRNA for the Chl *a/b*-protein on exposure of etiolated barley seedlings to light. Apel has found this light response to be regulated by phytochrome (1). We also have evidence which will be presented elsewhere that this response is regulated by phytochrome in *L. gibba*.

We have also examined the effects of the dark/light treatments on the *in vivo* synthesis of the Chl *a/b*-protein and have shown earlier (26) that the synthesis of this protein increases when plants are illuminated after 4 days of darkness. Such experiments with *Lemna* differ in some aspects from those with etiolated tissue. Developmental effects of light/dark transitions in higher plants are complex because of the need for photosynthesis, but *Lemna* can grow heterotrophically in the dark using sucrose as an energy source. By placing fully green tissue in the dark, we can observe

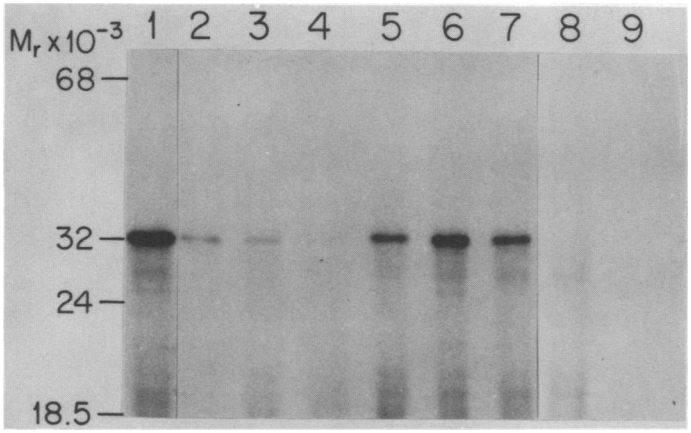

FIG. 4. Changes in translatable poly(A) RNA for the Chl *a/b*-protein in response to dark/light treatments. Fluorogram of immunoprecipitates of the Chl *a/b*-protein from aliquots of translation products containing nearly equal amounts of radioactivity after electrophoresis on a 12.5% polyacrylamide gel. Acid precipitable radioactivity in cpm × 10^{-3} in translation product aliquots used for immunoprecipitation: 1, 29.5; 2, 30.8; 3, 30.6; 4, 28.5; 5, 31.1; 6, 31.6; 7, 30.8; 8, 29.5. The RNA used for translations was isolated at the indicated times from plants grown under continuous white light, placed in the dark for 4 days, then returned to continuous light. 1, continuous light; 2, 1 day dark; 3, 2 days dark; 4, 4 days dark; 5, 4 days dark + 2 h light; 6, 4 days dark + 5 h light; 7, 4 days dark + 10.7 h light; 8, control immunoprecipitation of sample 1, using nonimmune serum; 9, immunoprecipitation from an aliquot of endogenous translation products (no added RNA).

Table I. *Relative Changes in Immunoprecipitable Chl a/b-Protein from Translation Products*

Figures given are the weights of paper in the area under the 32,000 peak in densitometer scans of the fluorogram shown in Figure 4.

Lane	Illumination	Weight
		mg
1	Continuous light	74.0
2	+ 1 day dark	8.1
3	+ 2 days dark	6.2
4	+ 4 days dark	1.6
5	4 days dark + 2 h light	23.4
6	4 days dark + 5 h light	53.0
7	4 days dark + 10.7 h light	26.3

the effect of light and darkness on tissue which is already green and contains substantial amounts of fully developed chloroplasts and the Chl *a/b*-protein. The tissue is not apparently senescing during the dark treatment (26), nor does the amount of the Chl *a/b*-protein decline appreciably during a 4-day dark treatment (Fig. 5). This figure shows samples of solubilized membranes obtained from equal fresh weights of tissue harvested before and after the dark treatment.

To examine the *in vivo* synthesis of the Chl *a/b*-apoprotein, it is important to establish that the [^{35}S]methionine incorporated into a protein of the electrophoretic mobility of the Chl *a/b*-apoprotein is actually associated with this polypeptide. I have confirmed this fact by using the antiserum to the Chl *a/b*-protein from *Chlamydomonas* to immunoprecipitate this protein from the solubilized membranes of *Lemna*. The appropriate amount of antiserum to use to recover the maximum amount of the protein was determined by titrating a constant volume of the solubilized membrane fraction from radioactively labeled plants with increasing amounts of antiserum and measuring the acid-precipitable radioactivity recovered in the immunoprecipitate. Figure 6 shows the [^{35}S]methionine-labeled immunoprecipitate. Lane A is the fluorogram of the

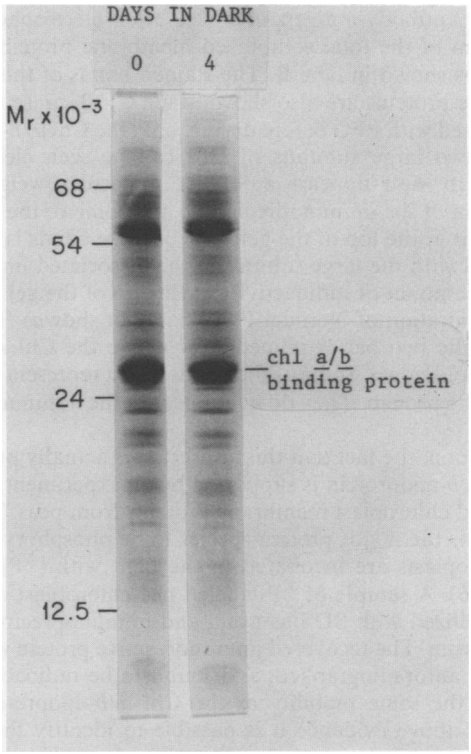

FIG. 5. Chl *a/b*-protein in dark-treated plants. The stained gel after electrophoresis of equal aliquots of the solubilized membrane fractions is shown. 0, plants grown in constant light; 4, plants transferred to darkness for 4 days.

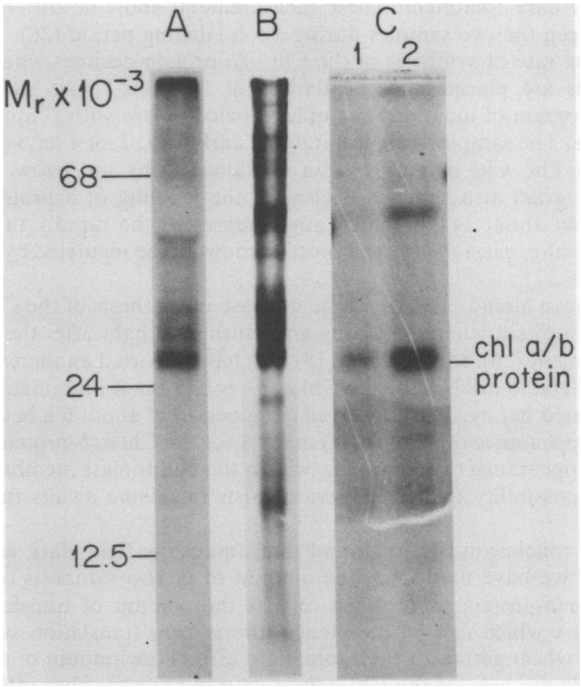

FIG. 6. Immunoprecipitation of Chl *a/b*-protein labeled *in vivo* in the light with [^{35}S]methionine for 1 h. A, fluorogram of immunoprecipitate after electrophoresis on a 12.5% polyacrylamide gel. B, fluorogram of solubilized membrane proteins used for the immunoprecipitation. C, dried gel stained with Coomassie Blue and impregnated with PPO. Lane 1 corresponds to B and was loaded with 5 μl of the sample. Lane 2 was loaded with 15 μl of the sample.

dissolved antibody-antigen complex after electrophoresis. The fluorogram of the total solubilized membrane proteins from the same gel is shown in lane B. The stained bands of this sample of membrane proteins are also shown (lane C). Because the gel was impregnated with PPO before drying, only the Chl *a/b*-apoprotein and the two large subunits of CF_1 can be seen clearly. Some radioactivity also appears at higher molecular weights in the fluorogram of the immunoprecipitate and some of the radioactivity remains at the top of the gel. One of these bands is apparently associated with the large subunit of the dissociated immunoglobulin. The amount of radioactivity at the top of the gel varies with the concentration of Nonidet P-40 (data not shown). The significance of the two bands immediately above the Chl *a/b*-protein bands is not known. Some of the bands might represent aggregated states of the protein. They do not appear in the nonimmune serum controls.

In addition, the fact that this antiserum is actually precipitating the Chl *a/b*-apoprotein is supported by an experiment with phosphorylated chloroplast membrane proteins from peas. The Chl *a/b*-protein is the major protein species to be phosphorylated when pea chloroplasts are incubated in the light with [^{32}P]orthophosphate (4–6). A sample of ^{32}P-labeled pea chloroplast membranes was solubilized with SDS as usual and immunoprecipitated with the antiserum. The recovered immunoreactive protein was electrophoresed, autoradiographed, and found to be radioactive and to run with the same mobility as the Chl *a/b*-apoprotein of pea. From the above evidence it is possible to identify the Chl *a/b*-apoprotein and its associated radioactivity incorporated during *in vivo* labeling with [^{35}S]methionine in *Lemna*.

To compare the synthesis rates between samples of plants it is important that the uptake and incorporation of the isotope does not differ between samples. We have previously (26) compared the uptake of [^{35}S]methionine and its incorporation into acid precipitable counts in plants grown in the light to plants given a 4-day dark treatment. These measurements show no differences between the two samples during a 5-h labeling period (26).

The rate of synthesis of the Chl *a/b*-protein declines when the plants are placed into the dark (Fig. 7). This figure shows a fluorogram of membrane samples labeled *in vivo* with [^{35}S]methionine. The samples were put into the dark for 1, 2, or 4 days (lanes 2–4). The Chl *a/b*-apoprotein is indicated by an arrow. This fluorogram also shows a decline in the labeling of a protein of mol wt about 34,000 which might represent the rapidly turning over chloroplast membrane protein known to be regulated by light (3, 19, 22).

I have already described the increase in synthesis of the Chl *a/b*-apoprotein when the plants are returned to light after the dark treatment (26). Muller *et al.* (18) also have reported an increase in translatable mRNA for the Chl *a/b*-protein upon illumination of etiolated barley. They observed a lag period of about 6 h between the appearance of the poly(A) mRNA for the Chl *a/b*-protein and the appearance of the protein within the chloroplast membranes. The possibility that a lag period exists in *Lemna* awaits further study.

In conclusion, I have found that, under the light/dark conditions we have used here, the amount of *in vivo* synthesis of the Chl *a/b*-protein in *L. gibba* reflects the amount of translatable mRNA which can be detected in the *in vitro* translation system from wheat germ. Furthermore, light affects the amount of translatable mRNA for the Chl *a/b*-protein relative to other mRNAs present in the plants, and hence the synthesis of this protein *in vivo*.

The rapidity of the changes in the translatable mRNA levels deserves some comment. Nearly 90% of the Chl *a/b*-protein mRNA detectable in the light-grown plants disappears after 1 day in the dark. This suggests a high rate of degradation or some sort of inactivation of this mRNA in the dark. These changes reported in the polysomal poly(A) RNA are also seen in the total cellular poly(A) RNA (24), and translation of poly(A)$^-$ RNA gives no indication of an increased poly(A)$^-$ Chl *a/b*-protein mRNA pool in the dark. It seems quite likely that light is affecting the rate of synthesis, processing, and/or degradation of the mRNA for the Chl *a/b*-protein as well as that for the small subunit of RuBP carboxylase (26). The use of *in vitro* translation only allows the determination of steady-state levels of the mRNAs. Further investigations of the kinetics of these mRNA changes and the exact process(es) being affected by light await the cloning of a suitable hydridization probe.

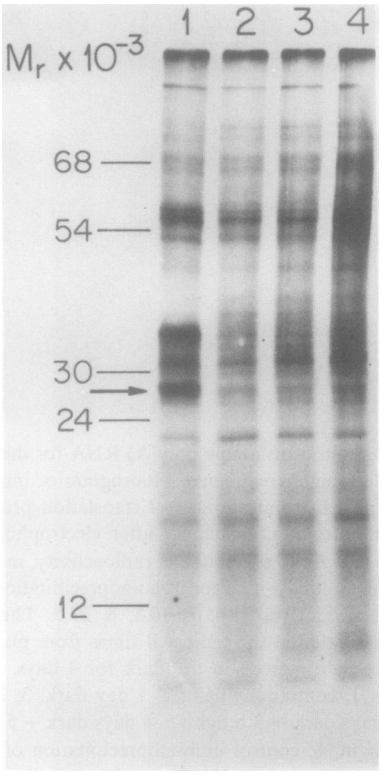

FIG. 7. Decline in Chl *a/b*-protein synthesis in the dark. Fluorogram of membrane fraction labeled *in vivo* after electrophoresis on a 12.5% polyacrylamide gel. Light-grown plants were placed in the darkness for the times indicated. The plants were labeled with [^{35}S]methionine for the last hour before harvesting. Times in darkness: 1, 0 h; 2, 21 h; 3, 45 h; 4, 96 h. The location of the Chl *a/b*-protein on the stained gel is marked with an arrow on the fluorogram. Acid precipitable radioactivity in cpm $\times 10^{-3}$ loaded onto lanes: 1: 89; 2: 88; 3: 88; 4: 89.

Acknowledgments—I thank Diane Kreman and Elaine Commiskey for their able technical assistance, Dale Hanson for the sample of erythrocyte poly(A) RNA, and Dr. John Bennett for preparing the sample of ^{32}P-labeled chloroplast membranes from pea as well as for helpful discussions and suggestions.

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